Pollen morphology and chromosome number of Vernonia rojasii (Vernonieae, Asteraceae)

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Abstract

Pollen morphology and chromosome number are reported for the first time in *Vernonia rojasii* CABRERA, an endemic species from central earaguay. The species presents a somatic chromosome number of 2n=4x=36, with the karyotype mostly composed by telocentric chromosomes. Pollen grains of *V. rojasii* are triporate, lophate, semitectate, with smooth crests on the muri. This pollen type has been previously called "E" and it is mostly present in Old World species of *Vernonia*. The basic chromosome number x=9 found in *V. rojasii* also indicates a probable close relationship with species of Africa and Asia, where this constitutes the more frequent number. In the meanwhile, almost all the American species have different types of tricolporate pollen which have been designated "A", "B", "C" and "D", and present basic chromosome numbers that vary from x=10 to x=19.

Introduction

Vernonia SCHREB. (Vernonicae, Asteraceae) contains approximately 800–1000 species widely distributed in southeastern Asia, Africa and America. The genus is mostly concentrated around two important centres of diversification, the tropical region of Africa and southern Brazil, where about 350–400 species occur (JONES 1977).

During the last years several authors have attempted to clarify the complex taxonomy of the genus (CABRERA 1944, KEELEY 1978, JONES 1979a, 1981, STUTTS 1988, ROBINSON 1988a, 1988b, 1989, 1990, 1992a, 1992b, 1992c, 1999). Most of the studies were based on vegetative morphology, while

relatively few analyses have considered the chemical composition, pollen morphology or chromosome number. The two latter have shown to be important features in the taxonomy of the genus. Pollen variations in *Vernonia* are numerous and striking (STIX 1960, KINGHAM 1976, KEELEY & JONES 1979) and some of these have been used taxonomically (JONES 1979a, 1981, ROBINSON 1988a, 1988b, 1989, 1990, 1992b, 1992c, 1999). General terminology used for pollen types in tribe Vernonieae dates initially from KEELEY & JONES (1979), who described six pollen classes to which posteriorly were incorporated several additional categories (ROBINSON 1990, 1992a, 1999). A total of ten different pollen types have been recognized in Vernonieae, some of them restricted to New World species while others are distinctive of Old World taxa (JONES 1979a, 1981, ROBINSON 1999).

Chromosome studies performed in the genus indicate certain differences between New and Old World species. The latter entities have basic chromosome numbers x=9 and x=10 (Jones 1979b), while the American taxa present a great diversity of numbers ranging from x=10 to x=19 (KEELEY & TURNER 1990, RUAS et al. 1991, DEMATTEIS & FERNÁNDEZ 1998).

In the present study are analysed for the first time the pollen morphology and chromosomes of *Vernonia rojasii* CABRERA, an endemic species from the Chaco region of Paraguay (CABRERA 1941). This species has remained practically unknown since its original description and relatively few collections have been made. In consequence, the relationships and taxonomic position of this taxon have not been appropriately discussed previously.

Material and Methods

The source of the examined specimens is the following: Paraguay. Dept. Alto Paraguay. 85 km E of Agua Dulce, on the road to Bahía Negra. F. MERELES et al. 6512. Voucher specimens have been deposited at the herbaria of Instituto de Botánica del Nordeste (CTES), Museo de La Plata (LP), Facultad de Ciencias Químicas of the Universidad Nacional de Asunción (FCQ) and the Smithsonian Institution (US).

Pollen material of dried specimens was obtained from the herbarium of Instituto de Botánica del Nordeste (CTES). The pollen grains were acetolysed according to the procedure suggested by ERDTMAN (1966). Due to the considerable exine thickness, sample fragments were bleached with nascent chlorine to allow a better observation. For light microscopy (LM) the pollen samples were mounted in glycerinjelly, sealed with paraffin and then examined with a Leitz Ortholux microscope. Permanent slides were deposited at the Palynological Laboratory of the Universidad Nacional del Nordeste (PAL-CTES). Pollen measurements such as polar diameter (P), equatorial diameter (E) and exine thickness are based on 30 grains.

For scanning electron microscopy (SEM) acetolysed pollen grains were first washed in 96 % alcohol and absolute alcohol, posteriorly sputtered with gold palladium and then observed in a JEOL 5800 LV - SEM. The terminology applied for pollen grain description in general follows ERDTMAN (1966), KREMP (1968) and PUNT et al. (1994).

Mitotic chromosome preparations were obtained from root-tips of germinating seeds. The rootlets were pretreated for about 4–4.5 hours in 8-hydroxyquinoline 0.002 M solution, fixed in lactic acid–absolute alcohol (1:5) and stained using the FEULGEN's technique.

Results

Pollen

LM: Pollen triporate, isopolar, radially symmetric; spherical or subspherical and middle sized (Fig. 1). P = 41 (44.6) 47 μ m, E = 42 (44.6) 49 μ m; P/E = 0.90–1.00. Pores circular, 4 μ m diameter. Exine 8.4 μ m wide, sexine thicker than nexine, semitectate-reticulate, reticulum homobrochate, rectimurate; muri narrow, ca. 1 μ m wide, simplicolumellate, columella irregularly branched in cross-sections; lumina 5–6 polygonal, 7–10.5 μ m diameter, lumina enclosing the pores large sized and longitudinally elongated.

SEM: Scanning analysis supports the LM observations and indicates the presence of brief protuberances, like spinules or smooth crests on the muri (Figs. 1–2).

Cytology

Vernonia rojasii showed a somatic chromosome number of 2n=36 (Fig. 3). The size of the chromosomes was relatively small, ranging from 0.76 to 2.05 μ m, with a mean length of 1.20 μ m. Due to the diminutive chromosome size, it was not possible to determine the karyotype composition, however a considerable proportion of pairs would be apparently telocentrics. The chromosome number found in *V. rojasii* indicates that it is a tetraploid species based on x=9.

Discussion

The pollen of Vernonia rojasii is triporate, lophate, semitectate, with smooth crests on the muri. This pollen type has been previously called "E" by KEELEY & JONES (1979) and is mostly present in species of the Eastern Hemisphere. In the meanwhile, most of the American species have different types of tricolporate pollen which have been named "A, "B", "C" and "D" (JONES 1979a). The pollen morphology of *V. rojasii* suggests that it would be more closely related to Old World members of *Vernonia* than to other New World taxa. The basic chromosome number x=9 found in *V. rojasii* also indicates a probable close relationship with species of Africa and Asia, where this constitutes the more frequent number. Nevertheless, *V. rojasii* can be distinguished from the latter on the base of its ploidy level (4x). In the Old World, most of the species are diploid and polyploidy is extremely uncommon (JONES 1979b).

The presence of telocentric chromosomes clearly separates *V. rojasii* from the remainder American members of Vernonieae that often have metacentric and submetacentric pairs (DEMATTEIS & FERNÁNDEZ 1998). Only some American species with base number x=16 present occasionally subtelocentric chromosomes, but telocentric pairs are generally absent in the genus *Vernonia* (DEMATTEIS 1998).

The basic chromosome number x=9 has been previously reported for *V. echitifolia* MART. ex DC., which would likely be closely related to *V. rojasii* taking into account its base number and pollen type. Like *V. rojasii*, this species has type "E" pollen and basic chromosome number x=9, with a karyotype composed of several acrocentric or telocentric pairs (DEMATTEIS & ROBINSON 1997). Despite its similar pollen type and chromosomes, both entities differ considerably in many other morphological features. *Vernonia echitifolia* is a densely pubescent shrub, covered by reddish glands on leaves, and presents sessile heads 8–13 flowered disposed in axillar location of reduced bracts. On the other hand, *V. rojasii* is a totally glabrous herb, without glands, having pedunculate heads, 90–100 flowered, which are arranged between leafy bracts.

Although the base number x=9 has not been reported for any other New World species, it may be present in some American taxa with type "E" pollen, since there is a clear relationship between pollen morphology and basic chromosome number in the tribe Vernonieae. The groups with tricolporate pollen show basic chromosome numbers x=17, x=16, x=15 and x=14, while the taxa that possess triporate pollen types have base x=8, x=9, x=10, x=11

and x=13 (ROBINSON 1992a). Two entities that may have x=9 are Vernonia brunneri (H. ROB.) CABR. and Pacourina edulis AUBL., which are the only other American taxa with type "E" pollen (ROBINSON 1992a, 1992b). From these, V. rojasii may be more closely related to V. brunneri, a species recently described for Paraguay and subsequently also discovered in Bolivia (ROBINSON 1999). This species is morphologically similar to V. rojasii, differing only in the leaf shape and size. Vernonia brunneri has oblong-ovate leaves, 2–7 cm long and 1–3 cm wide, while V. rojasii presents lanceolate leaves, which vary between 6–10 cm long and 0.5–2 cm wide.

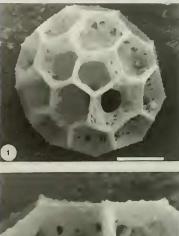
Additional chromosome studies, especially in *V. brunneri* and other taxa having triporate pollen, could provide a better understanding of the relationships between *V. rojasii* and the remainder American and African species of the tribe Vernonieae.

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Figs. 1–3. Vernonia rojasii.

 1-2. Pollen grain.
1. view showing pore. Scale=10μm.
2. details of the muri. Scale=2μm.
3. Somatic chromosomes, 2n=36. Scale=5μm.